

Investigation of the Toxic & Teratogenic Effects of GRAS Substances to the Developing  
Chicken Embryo      Guar Gum      No Date

**BB15**

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to the Developing Chicken Embryo  
GUAR GUM

Protocol:

Guar Gum was tested for toxic and teratogenic effects to the developing chicken embryo under four sets of conditions. It was administered in water as the solvent by the two routes at two stages of embryonic development: via the air cell at pre-incubation (0 hours) and at 96 hours of incubation, and via the yolk at 0 hours and at 96 hours using techniques that have been described previously (1, 2).

Groups of 10 or more eggs were treated under these four conditions at several dose levels until a total of ninety to one hundred eggs per level was reached for all levels allowing some hatch. Groups of comparable size were treated with the solvent at corresponding volumes and untreated controls were also included in each experiment.

After treatment, all eggs were candled daily and non-viable embryos removed. Surviving embryos were allowed to hatch. All hatched chicks and non-viable embryos were examined carefully for abnormalities (internally and externally) as well as toxic responses such as edema and hemorrhage. All abnormalities were tabulated.

Results:

The results obtained are presented in Tables 1 through 4 for each of the four conditions of the test.

Columns 1 and 2 give the dose administered in milligrams per egg and milligrams per kilogram, respectively (the milligrams per kilogram figure is based on an average egg weight of fifty grams). Column 3 is the total

number of eggs treated. Column 4 is the percent mortality i.e. total non-viable divided by total treated eggs. Column 5 is the total number of abnormal birds expressed as a percentage of the total eggs treated. This includes all abnormalities observed and also toxic responses such as edema, hemorrhage, hypopigmentation of the down and other disorders such as feather abnormalities, significant growth retardation, cachexia, ataxia or other nerve disorders. Column 6 is the total number of birds having a structural abnormality of the head, viscera, limbs, or body skeleton expressed as percentage of the total eggs treated. Toxic responses and disorders such as those noted for column 5 are not included.

Column 3 through 6 have been corrected for accidental deaths if any occurred. Included in these columns are comparable data for the solvent treated eggs and the untreated controls.

The mortality data in Column 4 have been examined for a linear relationship between the probit percent mortality versus the logarithm of the dose according to the procedures of Finney (3). The results obtained are indicated at the bottom of each table.

The data of Columns 4, 5, and 6 have been analyzed using the Chi Square Test for significant differences from the control background. Each dose level is compared to the control value and levels that show differences at the 5% level or lower are indicated by an asterisk in the table.

At hatchings, 3 chicks were removed at random from each level including control for skeletal clearing, weighing and fixing of bursa, spleen, liver and kidney. Tissues were processed, blocked in paraffin, sectioned, affixed to slides, and stained. Later these sections were examined for internal damage to the tissues.

Discussion:

Guar gum was tested at dose levels between 1 and 20 mg/kg for all four conditions of the test. The estimated LD-50 values for all the four treatments were as follows:

<u>Treatment</u>	<u>LD-50 Level</u>
Air cell treatment 0 hours	17.73 mg/kg (0.86 mg/egg)
Air cell treatment 96 hours	19.18 mg/kg (0.96 mg/egg)
Yolk treatment 0 hours	2.37 mg/kg (0.12 mg/egg)
Yolk treatment 96 hours	3.59 mg/kg (0.18 mg/egg)

Significantly higher mortality rates were observed in all treatments when Guar gum dose level was more than 2.5 mg/kg except for the air cell treatment at 96 hours. Guar gum produced significant mortality rates in air cell treatment at 96 hours only when the dose level was 20 mg/kg.

There were no teratogenic effects observed in any of the test conditions employed.

References:

1. McLaughlin, J., Jr., Marliac, J.-P., Verrett, M. Jacqueline, Mutchler, Mary K., and Fitzhugh, O. G., (1963) Toxicol. Appl. Pharmacol. 5, 760-770.
2. Verrett, M. J., Marliac, J.-P., and McLaughlin, J., Jr., (1964) JAOAC 47, 1003-1006.
3. Finney, D. J., (1964) Probit Analysis, 2nd Ed., Cambridge Press, Cambridge, Appendic I.

GUAR GUM  
AIR CELL 0 HOURS

DOSE		Number of Eggs	Percent Mortality *	Percent Abnormal	
mg/egg	mg/kg			Total	Structural
1.00	20.00	100	66.00 *	0.0	0.0
0.75	15.00	100	44.00 *	0.0	0.0
0.25	5.00	100	31.00 *	0.0	0.0
0.125	2.50	100	15.00	0.0	0.0
0.05	1.00	100	11.00	0.0	0.0
Water		100	14.00	0.0	0.0

\*Significantly different from solvent  $p \leq 0.05$

GUAR GUM  
AIR CELL 96 HOURS

DOSE		Number of Eggs	Percent Mortality*	Percent Abnormal	
mg/egg	mg/kg			Total	Structural
1.00	20.00	100	64.00*	0.0	0.0
0.75	15.00	100	26.00	0.0	0.0
0.25	5.00	100	17.00	0.0	0.0
0.125	2.50	100	9.00	0.0	0.0
0.05	1.00	100	12.00	0.0	0.0
Water	0.00	100	15.00	0.0	0.0

\*Significantly different from solvent  $p \leq 0.05$

GUAR GUM  
YOLK 0 HOURS

DOSE		Number of Eggs	Percent Mortality *	Percent Abnormal	
mg/egg	mg/kg			Total	Structural
1.00	20.00	100	100.00 *	0.0	0.0
0.75	15.00	100	99.00 *	0.0	0.0
0.25	5.00	100	82.00 *	0.0	0.0
0.125	2.50	100	59.00 *	0.0	0.0
0.05	1.00	100	49.00 *	0.0	0.0
Water		100	27.00	0.0	0.0

\*Significantly different from solvent  $p \leq 0.05$

GUAR GUM  
YOLK 96 HOURS

DOSE		Number of Eggs	Percent Mortality *	Percent Abnormal	
mg/egg	mg/kg			Total	Structural
1.00	20.00	100	100.00 *	0.0	0.0
0.75	15.00	100	89.90 *	0.0	0.0
0.25	5.00	100	77.00 *	0.0	0.0
0.125	2.50	100	51.00 *	0.0	0.0
0.05	1.00	100	41.00	0.0	0.0
Water		100	30.00	0.0	0.0

\*Significantly different from solvent  $p \leq 0.05$